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FOREWORD

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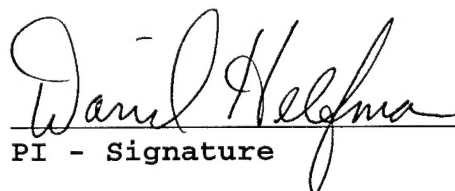
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INTRODUCTION:

The goal of this research program is to contribute to our understanding of the molecular basis of metastasis. The ability of a tumor cell to grow outside its local environment (metastasize) is a major problem in the development and therapeutic treatment of cancer. Adhesion of cells to the extracellular matrix (ECM) and neighboring cells plays a critical role in various cellular processes linked to transformation including differentiation, growth, motility and programmed cell death (apoptosis). Regulation of cell death is an essential component in the body's defense against the emergence of cancer. Attachment of cells to the ECM is important for the generation of signals that regulate normal cell proliferation and apoptosis. The loss of the requirement for cell-matrix interactions plays a critical role in the development of cancer. Recent experiments from our and other laboratories have shown that activation of actomyosin contractility is an essential step during adhesion-mediated signal transduction. In addition, this suggests a mechanism by which changes in cellular proteins involved in the regulation of myosin function lead to aberrant growth control by constitutively activating downstream signaling pathways. The experiments outlined in this grant will test the hypothesis that constitutive activation of actomyosin contractility contributes, in part, to the transformed phenotype and to the inability of transformed cells to undergo apoptosis. Specifically, we will determine if inhibition of myosin by pharmacological agents or overexpression of caldesmon and tropomyosin (TM), two cellular proteins that can regulate myosin function, results in activation of the apoptotic pathway in transformed cells. In addition, studies from our laboratory show that TM plays a causal role in the phenotype of ras-transformed fibroblasts (Gimona et al., 1996), and we have shown for the first time that nonmuscle caldesmon plays a key role in the regulation of actomyosin contractility and adhesion-mediated signaling in nonmuscle cells (Helfman et al., 1999). The experiments outlined in this grant will determine whether changes in TM and caldesmon in epithelial cells contribute to the transformed phenotype, as has been demonstrated for fibroblasts. We suggest that TM and caldesmon function as part of a cellular feedback mechanism to regulate various signal transduction pathways dependent on actomyosin contractility. Our studies will provide important new information on the role of these proteins in adhesion-dependent cell signaling and the potential of inhibiting signal transduction pathways dependent on actomyosin contractility as a therapeutic target and adjuvant for the treatment of breast cancer, as well as other cancers.

RESEARCH FINDINGS:

The observation that activation of actomyosin contractility is an essential step during adhesion-mediated signal transduction suggests a mechanism by which changes in cellular proteins involved in the regulation of myosin function in transformed cells will contribute to aberrant growth control. The experiments outlined for this grant in the original "**Statement of Work**" for the first 12 months were included in Specific Aim 1. Specifically to: (1) analyze the effects of treating cells with pharmacological agents to determine if inhibition of actomyosin contractility induces apoptosis and (2) test transient transfection of caldesmon on apoptosis. These experiments were designed to test the hypothesis that constitutive activation of actomyosin contractility contributes, in part, to the transformed phenotype and to the insensitivity of transformed cells to undergo apoptosis. Accordingly, during the first 12 months of this funding proposal we determined if inhibition of myosin results in activation of the apoptotic pathway in adhesion-independent cells. If activation of actomyosin contractility is a critical step in the pathway of adhesion-dependent suppression of apoptosis, then inhibiting myosin function will promote apoptosis. These studies provided important new information concerning the role of actin filaments and myosin in adhesion-dependent cell signaling and the potential of inhibiting signal transduction pathways dependent on actomyosin contractility as a therapeutic target and adjuvant for the treatment of breast cancer, as well as other cancers.

KEY RESEARCH ACCOMPLISHMENTS:

The experiments conducted during the current funding period were designed to determine if activation of actomyosin contractility plays a role in intracellular signaling following cell-ECM interactions and thereby prevents apoptosis. Accordingly, if loss of cell-ECM interactions results in a decrease in actomyosin contractility and thereby activates the apoptotic pathway in normal cells, then inhibition of myosin itself should lead to apoptosis. In addition, if transformed cells are able to exhibit adhesion-independent cell growth because of constitutive activation of actomyosin contractility, then inhibiting myosin II function should effect cell growth and cell survival. Accordingly, we studied the effects of altering myosin II function in normal MDCK cells and a variety of transformed human mammary epithelial cells. The normal epithelial cell line exhibits the same pattern of TM expression as primary epithelial. By contrast, the various ductal and adenocarcinoma cell lines exhibit a loss of specific high molecular weight TMs already implicated in transformation of fibroblasts. These cells provide a useful model to study the relationships between changes in TM expression and cell transformation for experiments outlined in Specific Aims 2 and 3 during years 2 and 3 of this grant.

We used two different compounds that inhibit myosin II, namely Butanedione monoxime (BDM) and ML-7. BDM inhibits the ATPase activity of myosin and ML-7 blocks myosin light chain kinase and thereby inhibits myosin II contractility. Apoptosis was assayed by staining cells with DAPI and viewing the nuclei or by analysis of DNA degradation. We also assayed for other known markers of apoptosis including staining cells for annexin V, and processing of caspases. For the studies we continually obtain the advice of Drs. Michael Hengartner, Yuri Lazebnick, Scott Lowe, at Cold Spring Harbor Laboratory, who are experts in apoptosis.

The second method we are using to investigate the role of cell contractility in adhesion-dependent signaling and apoptosis is to study the effects of caldesmon. We have recently found that overexpression of nonmuscle caldesmon in fibroblasts blocks cell contractility (Helfman et al., 1999). Thus caldesmon is a potent and specific tool that can regulate myosin-II contractility. We are using transient transfection of epitope-tagged constructs into MDCK cells and various human mammary epithelial cells. Cells are fixed and stained at 24, 48 and 72 hours post-transfection. and analyzed by immunostaining of cells transfected with tagged-isoforms and cells stained for markers to assess the apoptotic response.

The results of our data indicate that agents that inhibit myosin II function lead to programmed cell death in epithelial cells. Thus, actomyosin contractility plays a critical role in the generation of signals required for the prevention of apoptosis in epithelial cells.

REPORTABLE OUTCOMES:

This funding provides research support for a doctoral student and postdoctoral fellow.

CONCLUSIONS:

Our current studies are in agreement with our hypothesis concerning the role that myosin II function plays in adhesion-dependent cell growth and in the apoptotic response of cells. In this regard it is interesting to note that Rho plays a role in both transformation (Qiu et al., 1995) and metastasis (Yoshioka et al, 1998). Rho is a downstream effector of the ras pathway is known to stimulate the actomyosin system and this is thought to play a direct role in invasion of tumor cells (Yoshioka et al., 1998). While this role is believed to involve increase motility of tumor cells, it is also possible that activation of contractility also activates signal transduction pathways associated with adhesion-dependent cell growth, that would antagonize the normal apoptotic pathway and lead to survival of tumor cells outside their normal environment. Thus our studies have provided important new information on the role of actomyosin contractility in adhesion-dependent cell signaling and the potential

of inhibiting signal transduction pathways dependent on actomyosin contractility as a therapeutic target and adjuvant for the treatment of breast cancer, as well as other cancers. Helfman, David, M.

REFERENCES:

- Gimona, M., Kazzaz, J., and Helfman, D.M.** (1996). Forced expression of tropomyosin 2 and 3 in vi-Ki-ras-transformed fibroblasts results in distinct phenotypic effects. *Proc. Natl. Acad. Sci. USA* 93, 9618-9623.
- Helfman, D.M., Levy, E., Berthier, C., Shtutman, M. Elbaum, and Bershadsky, A.D.** (1997). Block of cell contractility by caldesmon interferes with the rho-dependent formation of focal adhesions. *ASCB Meeting, Washington, D.C.* *Mol. Biol. Cell* 8, 415a.
- Helfman, D.M., Levy, E.T., Berthier, C., Shtutman, M. Elbaum, M., and Bershadsky, A.D.** (1999). Caldesmon inhibits nonmuscle cell contractility and interferes with the formation of focal adhesions. *Mol. Biol. Cell* 10, 3097-3112.
- Qiu, R.-G., Chen, J., McCormick, F., and Symons, M.** (1995). A role for rho in ras transformation. *Proc. Natl. Acad. Sci. USA* 92, 11781-11785.
- Yoshioka, K., Matsumura, F., Akedo, H., and Itoh, K.** (1998). Small GTP-binding protein rho stimulates the actomyosin system, leading to invasion of tumor cells. *J. Biol. Chem.* 273, 5146-5154.